(b) contacting the P1-TP complex of (a) with an exonuclease deficient deoxyribonucleotide (DNA) polymerase enzyme under conditions that promote extension of the P1 with the TP as template thereby forming an extended segment (ES) of P1; and

(c) detecting the extended P1

thereby detecting an SNP in said target polynucleotide.

REMARKS

Claims 1-30 are pending in the case. Claims 16, 17 and 30 have been withdrawn from examination. Claim 15 has been objected. The other claims have been rejected. New claim 31 has been added.

Rejection Under 35 U.S.C. §102

Claims 1 and 9 were rejected under section 102 as anticipated by Eyal et al (U.S. Patent 5,710,028). In response, Applicant notes that this section of the Office Action recites teachings not by Eyal et al but, rather, by Chee et al (U.S. Patent 6,355,431). For purposes of response, Applicant presumes that it is the Chee et al reference that is being relied upon but will respond to both.

Chee discloses single base extension using a terminating nucleotide so that only one base is added and only if this base is complementary to the corresponding

base on the target strand (see column 16, lines 37-39, of Chee). Conversely, Applicant is extending from the match to form an extended primer (see application at page 8, line 9, where Applicant teaches "extending only from a matched nucleotide") thereby showing that single base extension is disclaimed since the method of the invention calls for extending from the matched nucleotide. In addition, at page 12, lines 6-8, of the application, a preferred embodiment is at about 40 to 50 nucleotides extension, but at least about 40 nucleotides. Thus, single base extension is not encompassed by the application. This is especially true in light of the added steps of claim 2, wherein the target strand is removed and a P2 primer is hybridized to the extended portion of P1 followed by rolling circle amplification.

In view of the foregoing, Chee et al does not anticipate the invention of claim 1.

For the same reasons, Eyal et al does not anticipate the claimed invention. The rejection refers to Eyal et al at column 11, lines 28-38, showing contacting an allele specific oligonucleotide primer with a target polynucleotide, but in Eyal et al it shows clearly that the base to be identified is the first unpaired base of the target that is 5'- of the base that binds to the 3' end of the primer oligonucleotide. This is reiterated by Eyal et al at column 13, lines 33-42, where the formation of single base extensions is again described. Conversely, in claim 1 of the application it is the 3'-terminal base of the primer that may or may not match the corresponding or adjacent base of the target strand. Thus, this element of the claim is not met and Eyal et al does not anticipate claim 1.

In addition, Eyal et al is relied on to show use of hybridized oligonucleotides to detect the extended primer (citing column 7, lines 27-67). Applicant contends that such reliance is misplaced because the cited disclosure again reveals the use of single base extensions (see column 7, at lines 34-35). Thus, Eyal et al cannot be disclosing

use of oligonucleotides to detect the extended primer because the primer is only extended by 1 nucleotide. Thus, what is being taught is the use of a detection oligonucleotide complementary to the non-extended portion of the primer. Thus, again, Eyal et al does not anticipate the claimed invention.

The foregoing argument is re-enforced by the amendment to claim 1 wherein the extension of P1 is detected by hybridizing P2 to the extended P1. In addition, the process of claim 1 achieves extension of P1 beyond the point of match of the nucleotides of the polymorphism whereas in Eyal et al and in Chee et al it is the matched nucleotide that is added (and using a poison pill to prevent any further extension. Thus, claim 1, with or without amendment is not anticipated by either Eyal et al or Chee et al, separately or when taken together.

Rejection Under 35 U.S.C. §103

Initially, Applicant notes that the Office Action makes mention of joint inventorship. Applicant believes that there is only one named inventor and therefore 37 C.F.R. 1.56 is not applicable.

Claims 1-14 and 18-25 were rejected under section 103(a) as being unpatentable over Eyal et al (U.S. Patent 5,710,028) in view of Chee et al (U.S. Patent 6,355,431).

Applicant responds by reiterating the argument regarding novelty, supra. In addition, Applicant notes that both Eyer et al and Chee et al teach use of single base extension. Importantly, both include an extension terminating unit to prevent extension beyond a single nucleotide, which nucleotide is added or not added to the primer, depending on the adjacent base of the target strand, and where added there

is a "poison pill" to terminate further extension. In the present invention, this base is adjacent to the 3'-end of the primer so that P1 either matches or does not match the suspected polymorphism and only extension of the primer beyond the match is achieved. This result is inherent in the claim language because the point of match or mismatch is recited to lie at the 3'-end of the primer.

In Eyal et al, for example, what is taught is the use of 4 different reactions, each with a different nucleotide and each containing a "poison pill" to prevent more that a one unit extension. In addition, reliance on Eyal et al at column 7, lines 27-67, does not support the rejection because it recites use of four single nucleotide reactions (lines 34-35) with a poison pill (here, Eyal et al recites the dideoxynucleotide-biotin of lines 65-66 as extension terminating group with the biotin used for detection). Thus, Eyer et al teaches detection of single base extension (for example, Figures 1 and 2) or simultaneous detection of multiple mutations, each using a single base extension of the corresponding mutation (see Figures 4 and 5).

In addition, as amended, claim 1 recites use of the P2 primer hybridizing to the extended portion of P1 so as to detect extension (which extension is not taught by either Eyal or Chee, separately or together). Further, Chee's reciting of the DNA polymerase for extension of the primer (see Office Action at page 5, citing Chee et al at column 16, starting line 49) is disingenuous in that this very portion of Chee et al teaches use of a nucleotide analog, defined as a nucleotide that is chain terminating (see column 16, at lines 52-55). Clearly, Chee again teaches only single base extension, regardless of what type of polymerase enzyme is being used. If the primer is being extended by only a single base, any subsequent determination of extension using hybridization with another oligonucleotide cannot involve the extended segment because that is always no more than 1 base in length.

The argument of the rejection that Chee et al also teach use of rolling circle

amplification (RCA) is again unavailing. There, Chee teaches only two embodiments of this detection method. The first involves formation of the circle by circularizing a probe and then ligating it, thereby producing an amplification target circle with the target strand hybridized to the probe and acting as primer for RCA (see column 19, lines 20-21). The second involves hybridizing a probe to a target strand, then hybridizing a second probe and then ligating the probes (see column 19, lines 33-36). In either case, the polymorphism is determined by whether or not the ligation occurs, with details added in columns 20 and 21.

Conversely, Applicant's claimed process involves no ligation of any kind. Only claim 2 recites RCA and no ligation is utilized. Applicant fails to see how the claimed processes of either claims 1 or 2, or any claims dependent therefrom, can be rendered obvious by references reciting only methods that rely on steps in addition to the steps taught by Applicant. If, in fact, Applicant teaches a way to identify single nucleotide polymorphisms without the use of a ligation step then that in itself should be patentable.

Applicant notes that claim 1 was amended to recite use of the P2 primer as a means of detecting extension of P1, which extension is inherently beyond the point of match or mismatch, and which amendment is supported by the disclosure of Figure 1, Figure 2, and the description of P2 at page 16, lines 4-5. In addition, the use of a bipolar P2 primer in amended claim 2 is supported by the figures and the description of P2 at page 16, line 8, where the primer has two 3'-ends (as in amended claim 3), as well as at page 22, lines 4-16. Thus, the claim amendments are amply supported by the disclosure of the application as filed.

The rejection also relies on the Ishikawa et al (1995) paper as teaching the use of a one base mismatch to improve specificity. Because this reference is offered to show obviousness, the motivation to combine it with the others is important.

Applicant respectfully contends that there is no such motivation because the methodologies are different. Ishikawa et al is doing PCR studies and uses the mismatch at the second position from the 3'-end to improve annealing during thermocycling (see page 316, column 2, paragraph beginning "Figure 2 shows..." and also see the description of Figure 2 on page 317. Conversely, Applicant is not relying on a mismatch near the 3' terminus to increase the specificity of PCR amplification but to increase the specificity of primer extension (showing allele discrimination) under isothermal conditions. Consequently, Ishikawa et al is inapposite.

New Claim

The Examiner objected to claim 15 on grounds that it contained sequences other than elected sequence 13 but that the claim would be allowable if limited to SEQ ID NO: 13. In response, Applicant has added new claim 31, which recites original claim 1 with P1 comprising the sequence of SEQ ID NO: 13 (see the end of step (a)). In addition, the term "target polynucleotide" has been added to the preamble and a closing phrase was added after the final step. No other modifications were made.

Information Disclosure

Applicant is now in receipt of references cited in an International Search Report for a PCT application corresponding to the present application. Applicant has included copies of these references along with the present response (including an IDS and Form 1449). No additional fee has been included because the Search Report was

mailed to Applicant's agent by the European Patent Office on 18 October 2002, several months after the date of the current Office Action.

The Commissioner is authorized to charge payment of any additional filing fees required under 37 CFR 1.16 associated with this communication or credit any overpayment to Deposit Account No. 03-0678.

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Alan J. Grant, Esq. **Date**

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AMENDED SPECIFICATION

The paragraph at page 36, lines 4-10, was amended as follows:

In a separate embodiment of the invention, it is possibly to detect multiple single nucleotide polymorphisms (i.e., multiple alleles) simultaneously through the process of multiplexing. Thus, at least 4 different allele-specific oligonucleotides (P1) can be employed to detect any one of 4 possible point mutations at the given site on the target DNA. Each such allele-specific oligonucleotide would possess a different 3'-terminal nucleotide residue. ~~For~~ (for example, using the method of Figure 2).

AMENDED CLAIMS

1. (Once Amended) A process for detecting a single nucleotide polymorphism (SNP) in a target polynucleotide comprising:

(a) contacting one or more allele specific oligonucleotide primers (P1) with one or more target polynucleotides (TP), wherein said target polynucleotide possesses a first portion that is complementary to a second portion located on said P1 at or near one end thereof but wherein the terminal nucleotide, and third nucleotide from the terminal nucleotide, at said end of said P1 may not be complementary to the corresponding nucleotide of said target polynucleotide, and wherein such contacting occurs under conditions that promote hybridization between the first and second portions thereby forming an P1-TP complex;

(b) contacting the P1-TP complex of (a) with an exonuclease deficient deoxyribonucleotide (DNA) polymerase enzyme under conditions that promote extension of the P1 with the TP as template thereby forming an extended segment (ES) of P1;

(c) detecting the extended P1 by removing the target polynucleotide from the complex formed in step (b) and contacting a primer oligonucleotide (P2) with the extended P1, wherein P2 comprises a portion that hybridizes to the extended segment of P1 and not to the non-extended portion of P1 under conditions promoting such hybridization; and

(d) detecting said hybridization of P2 and extended P1;

whereby said hybridization indicates extension of P1 thereby detecting an SNP in the target polynucleotide.

2. (Once Amended) A process for detecting the hybridization of P2 and extended P1 ~~of~~ in the process of claim 1 comprising the ~~further~~ steps of:

~~(d) removing the target polynucleotide (TP) from the complex formed in step (b);~~

~~(e) contacting primer oligonucleotide (P2) with the extended P1, wherein the primer oligonucleotide comprises a first segment complementary to at least a portion of the extended segment (ES) formed in step (b) and a second segment that includes the 3'-terminus of said primer oligonucleotide (P2) under conditions promoting hybridization of P2 and the extended P1 (EP1) to form an EP1-P2 complex;~~

~~(f) contacting an amplification target circle (ATC) with the EP1-P2 complex under conditions that promote hybridization between the amplification target circle and the P2 portion of said EP1-P2 complex to form an EP1-P2-ATC complex; and~~

~~(g) contacting DNA polymerase with the EP1-P2-ATC complex under conditions that promote replication of the amplification target circle,~~

~~wherein said replication of the ATC results in the formation of tandem sequence DNA (TS-DNA) thereby indicating the extension of P1.~~

(a) contacting an amplification target circle (ATC) with the hybridized P2 and extended P1 of claim 1 wherein said P2 comprises a first portion that hybridizes to the extended segment of P1 and not to the non-extended portion of P1 and a second portion that hybridizes to said ATC but not to P1, wherein P2 is a bipolar oligonucleotide and under conditions promoting hybridization of the ATC to P2 to form hybridized ATC-P2;

(b) contacting said hybridized ATC-P2 with a DNA polymerase under conditions promoting extension of P2 to produce rolling circle amplification of said ATC and thereby generating tandem sequence DNA (TS-DNA); and

(c) detecting production of said TS-DNA thereby detecting hybridization of P2 and extended P1.

3. (Once Amended) The process of claim 2 wherein ~~the target polynucleotide is derived from genomic DNA~~ P2 comprises two 3'-ends.

4. (Once Amended) The process of claim 2 wherein the ~~DNA~~ target polynucleotide is derived from genomic DNA.

7. (Once Amended) The process of claim ~~2~~ 4 wherein the target ~~DNA~~ polynucleotide is a mixture of human and non-human genomic DNA.

8. (Once Amended) The process of claim 2 wherein the DNA polymerase of step ~~(g)~~ (b) is an enzyme selected from the group consisting of bacteriophage φ29 DNA polymerase, phage M2 DNA polymerase, phage φ-PRD1 DNA polymerase, VENT® DNA polymerase, Klenow fragment of DNA polymerase I, T5 DNA polymerase, PRD1 DNA polymerase, T4 DNA polymerase, *E. coli* DNA polymerase III holoenzyme, Tts polymerase and T7 DNA polymerase.

10. (Once Amended) The process of claim 2 wherein the DNA polymerase of step ~~(g)~~ (b) is exonuclease deficient.